

sufficiently slow that the metabolic rate of sperm is decreased, to provide a cooled [sperm] first sample solution, maintained for a period of time at said first temperature;

- b) adding a solution comprising glycerol to [the] said cooled [sperm] first sample solution; [and]
- c) mixing said glycerol and said cooled first sample solution together to comprise a second sample solution;
- [c)] d) lowering the temperature of [freezing] said [cooled sperm] second sample solution to a second temperature for a sufficient period of time to equilibrate glycerol and sperm; and
- e) freezing said second sample solution to thereby provide a frozen sperm sample, such that the sperm is preserved[.];

claim 2 → wherein said first sample solution does not contain glycerol;

new limitation → wherein said second sample solution further comprises an antibiotic compound;

claim 2 → { wherein said sperm sample is cooled to said first temperature and maintained at said first temperature for 4 hours to 21 hours; wherein said second sample solution is allowed to freeze at said second temperature.

\$U 4854
(2, 5, 10, 15)

Please Cancel Claim 2

3. (Amended) The method of claim 1, wherein [the] said second sample solution [cryoprotectant buffer] comprises about 10% to about 30% egg yolk.
4. (Amended) The method of claim 1, wherein [the] said second sample solution comprises [concentration of glycerol in the sample after the addition of the glycerol solution is about] 5% to about 10% glycerol.
5. (Amended) The method of claim 1, wherein [the] said sperm sample is obtained from a mammal.

6. (Amended) The method of claim 1, wherein [the] said first temperature is between [about] 0°C and [to about] 10°C.

Please Cancel Claim 7

8. (Amended) The method of claim 1, wherein [the sperm] said first sample solution is cooled at a rate of between 0.2°C [to about] and 0.5°C per minute to [the] reach said first temperature.

9. (Amended) The method of claim 1, wherein [the sperm] said first sample solution is cooled to the first temperature over the course of [about] 1.5 to [about] 4 hours.

10. (Amended) The method of claim 1, wherein [the] said second temperature is between [about] -40°C [to about] and -100°C.

11. (Amended) The method of claim 1, wherein the sample is maintained at [the] said second temperature for between [about] 7 minutes and [to about] 20 minutes.

12. (Amended) The method of claim 1, wherein the method further comprises storing the frozen sperm sample at a third temperature of [about] between -190°C [to about] and -200°C.

13. (Amended) A method of preserving sperm comprising:
- a) combining a sperm sample with a first cryoprotectant buffer to form a first cryoprotectant solution;
 - b) cooling [sperm] said first cryoprotectant solution to a first temperature such first temperature being between [about] 2°C [to about] and 10°C at a rate sufficiently slow that the metabolic rate of sperm is decreased to produce cooled sperm;

- c) lowering the temperature of said first cryoprotectant solution to [the cooled sperm at] a second temperature between [about] -60°C and [to about] -90°C wherein said first cryoprotectant solution can freeze; and
- d) storing [the frozen sperm] said frozen first cryoprotectant solution in liquid nitrogen at a third temperature[.];
wherein said sperm sample is cooled to said first temperature and maintained at said first temperature for 4 hours to 21 hours;
wherein said first cryoprotectant solution further comprises an antibiotic compound.

new
line: 1-2-20

14. (Amended) The method of claim 13, wherein [the] said first cryoprotectant buffer comprises [about] 5% to [about] 10% glycerol weight to volume of said cryoprotectant buffer.

from the beginning

Please Cancel Claim 15

16. (Amended) The method of claim 13, wherein [the] said sperm sample is obtained from a mammal.
17. (Amended) The method of claim 13, wherein [the] said sperm sample is maintained at [the] said first temperature for between [about] 4 hours [to about] and 21 hours.
18. (Amended) The method of claim 13, wherein [the] said [sperm] first cryoprotectant solution is cooled at a rate of between [about] 0.2°C and [to about] 0.5°C per minute to reach said [the] first temperature.
19. (Amended) The method of claim 13, wherein [the sperm] said first cryoprotectant solution is cooled to [the] said first temperature over the course of [about] 1.5 hours to [about] 4 hours.

20. (Amended) The method of claim 13, wherein a second cryoprotectant buffer is added to said first cryoprotectant solution [the sample] after [the] said first cryoprotectant solution has been [sperm is] cooled to [the] said first temperature, [and] but before [the sperm is] said first cryoprotectant solution has been further cooled to [the] said second temperature or frozen.

no
whipmark
but
intended for
glycerol →

21. (Amended) The method of claim 20 wherein [the] said second cryoprotectant buffer comprises [about] 5% to [about] 10% glycerol.

22. (Amended) The method of claim 13, wherein [the] said second temperature is at least [about] -80°.

23. (Amended) The method of claim 13, wherein said first cryoprotectant solution [the sample] is maintained at [the] said second temperature for between [about] 7 minutes [to about] and 20 minutes.

A

24. (Amended) A method of [providing] preserving sperm comprising:

- a) providing a sample comprising sperm;
- b) isolating sperm from [the] said sample;
- c) combining said isolated sperm with a first cryoprotectant buffer to form a first sample solution [which lacks glycerol];
- d) cooling said [sperm] first sample solution to a first temperature [of about] between 2°C [to about] and 8°C at a rate of [about] between 0.2°C and [to] 0.5°C per minute to produce [cooled sperm] said first sample solution at said first temperature;
- e) adding a second cryoprotectant buffer [which contains] comprising glycerol to form a second sample solution;

- f) maintaining [the cooled sperm at the first temperature] said second sample solution at said first temperature for a [duration] period of between [about] 4 hours [to about] and 21 hours;
- g) [freezing the cooled sperm] lowering the temperature of said second sample solution by cooling said second sample solution to [at] a second temperature of [about] between -60°C [to about] and -90°C for a time of between about 10 minutes to about 15 minutes;
- h) allowing said second sample solution to freeze at said second temperature;
- [h)] i) storing [the frozen sperm] said second sample solution at a third temperature of between [about] -180°C [to about] and -220°C for a desired time period; [and]
- i) thawing said sperm, to thereby provide sperm for use [.]; and

wherein said first sample solution does not contain glycerol;

wherein said second sample solution further comprises an antibiotic compound.

25. (Amended) The method of claim 24, wherein the sperm are thawed for about 90 seconds in a water bath at about 37°C prior to use.
26. (Amended) A method of making an animal, comprising fertilizing an oocyte with sperm preserved by the method of claims 1, [claim] 13, or [claim] 24.

Please add claims 27 – 44

27. (New) A method of preserving sperm comprising:

- a) cooling a first sample solution which includes sperm to a first temperature sufficient to protect sperm from glycerol toxicity, at a rate sufficiently slow

that the metabolic rate of sperm is decreased, to provide a cooled first sample solution, maintained for a period of time at said first temperature;

- b) adding a cryoprotectant solution to said cooled first sample solution;
- c) mixing said cryoprotectant solution and said cooled first sample solution together to comprise a second sample solution;
- d) lowering the temperature of said second sample solution to a second temperature for a sufficient period of time to equilibrate said cryoprotectant solution and sperm comprising said second sample solution; and
- e) freezing said second sample solution to thereby provide a frozen sperm sample, such that the sperm is preserved;

wherein said cryoprotectant solution is further comprised of egg yolk, fructose, citric acid, Tris buffer; and an antibiotic compound;

wherein said sperm sample is cooled to said first temperature and maintained at said first temperature for 4 hours to 21 hours.

- A
- 28. (New) The method of claim 27 wherein neither said first sample solution or said second sample solution contain glycerol. ?
 - 29. (New) The method of claim 27 wherein said egg yolk comprises 10% to 30% weight to volume of said cryoprotectant solution.
 - 30. (New) The method of claim 27 wherein said egg yolk comprises 20% weight to volume of said cryoprotectant solution.
 - 31. (New) The method of claim 27 wherein said fructose comprises at least 1% weight to volume of said cryoprotectant solution.
 - 32. (New) The method of claim 27 wherein said citric acid comprises at least 1.5% weight to volume of said cryoprotectant solution.

33. (New) The method of claim 27 wherein said antibiotic compound is selected from a group consisting of:

- a) tylosin;
- b) gentamicin;
- c) lincospectin; and
- d) spectinomycin.

34. (New) A sperm cryoprotectant solution comprising egg yolk, fructose, citric acid, Tris buffer; and an antibiotic compound, wherein said egg yolk comprises 10% to 30% weight to volume of said cryoprotectant solution; wherein said fructose comprises at least 1% weight to volume of said cryoprotectant solution; and wherein said citric acid comprises at least 1.5% weight to volume of said cryoprotectant solution.

35. (New) The sperm cryoprotectant solution of claim 34 wherein said egg yolk comprises 20% weight to volume of said cryoprotectant solution.

36. (New) The sperm cryoprotectant solution of claim 34 wherein said fructose comprises 1% weight to volume of said cryoprotectant solution.

37. (New) The sperm cryoprotectant solution of claim 34 wherein said citric acid comprises 1.5% weight to volume of said cryoprotectant solution.

38. (New) The sperm cryoprotectant solution of claim 34 wherein said antibiotic compound is selected from a group consisting of:

- a) tylosin;
- b) gentamicin;
- c) lincospectin; and

d) spectinomycin.

39. (New) A sperm cryoprotectant solution comprising glycerol, egg yolk, fructose, citric acid, Tris buffer; and an antibiotic compound, wherein said glycerol comprises 5% to 10% weight to volume of said sperm cryoprotectant solution; wherein said egg yolk comprises 10% to 30% weight to volume of said sperm cryoprotectant solution; wherein said fructose comprises at least 1% weight to volume of said sperm cryoprotectant solution; and wherein said citric acid comprises at least 1.5% weight to volume of said sperm cryoprotectant solution.

40. (New) The sperm cryoprotectant solution of claim 39 wherein said glycerol comprises 7% weight to volume of said cryoprotectant solution.

41. (New) The sperm cryoprotectant solution of claim 39 wherein said egg yolk comprises 20% weight to volume of said cryoprotectant solution.

42. (New) The sperm cryoprotectant solution of claim 39 wherein said fructose comprises 1% weight to volume of said cryoprotectant solution.

43. (New) The sperm cryoprotectant solution of claim 39 wherein said citric acid comprises 1.5% weight to volume of said cryoprotectant solution.

44. (New) The sperm cryoprotectant solution of claim 39 wherein said antibiotic compound is selected from a group consisting of:

a) tylosin;

b) gentamicin;

c) lincospectin; and

d) spectinomycin.